

Amendments to the Specification:

Please enter the attached Sequence Listing into the Specification.

Attachments: Nucleotide Listing in written form (16 pages)

Diskette containing the Sequence Listing in computer readable form

Please replace the paragraph 1 of page 1 entitled "Reference to Related Applications" with the below rewritten paragraph:

Cross Reference To Related Applications

[0001] This application is a United States national phase filing under 35 U.S.C. § 371 of International Application No. PCT/SG2003/000205, filed August 29, 2003, which claims the benefit of U.S. Provisional Application No. 60/406,659, filed August 29, 2002, the content of which is herein incorporated by reference.

Please replace paragraph no. 0032 with the following amended paragraph.

[0032] The recombinant nucleic acid may be DNA or RNA. In one embodiment the recombinant nucleic acid is DNA comprising a gene encoding a first signal peptide operably linked to a gene encoding an allergen wherein the first signal peptide mediates the translocation of the allergen once expressed in the cell, into the endoplasmic reticulum. The gene encoding a first signal peptide may be any sequence that encodes an amino acid sequence that acts as a signal for protein folding machinery within the cell to direct the allergen to which the amino acid sequence is linked, to the endoplasmic reticulum. For example and without limitation, the first signal peptide may be the N-terminal signal sequence from the gene for LAMP-1, human tissue plasminogen activator (see for example ~~SEQ ID NO: 49~~ SEQ ID No. 48), lysosomal membrane protein LIMP-II (see for example ~~SEQ ID NOS: 8, 10, 12, 28, 30, 32~~ SEQ ID Nos. 7, 9, 11, 27, 29, and 31), (CD4+ T Cells Induced by a DNA Vaccine: Immunological Consequences of Epitope Specific Lysosomal Targeting. Fernando Rodriguez, Stephanie Harkins, Jeffrey M.

Redwine, Jose M. De Pereda, And J. Lindsay Whitton. JOURNAL OF VIROLOGY, Vol. 75(21): 10421- 10430.2001; The Residues Leu(Ile)⁴⁷⁵-Ile(Leu, Val, Ala)⁴⁷⁶, Contained in the Extended Carboxyl Cytoplasmic Tail, Are Critical for Targeting of the Resident Lysosomal Membrane Protein LIMP II to Lysosomes. Ignacio V. Sandoval Juan J. ArredondoS, Jose Alcalde, Alfonso Gonzalez Noriegall, Joel Vandekerckhove, Maria A. Jimenezll, and Manuel Rico. The Journal of Biochemistry, Vol. 269(9): 6622-6631,1994; Targeting of Lysosomal Integral Membrane Protein LIMP II THE TYROSINE-LACKING CARBOXYL CYTOPLASMIC TAIL OF LIMP II IS SUFFICIENT FOR DIRECT TARGETING TO LYSOSOMES. Miguel A. Vega, Fernando RodriguezSV, Bartolome Segui, Cannela Calesll, Jose Alcalde, and Ignacio V. Sandoval. THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 266(25): 16269-16272,1991; Cloning, Sequencing, and Expression of a cDNA Encoding Rat LIMP 11, a Novel 74-kDa Lysosomal Membrane Protein Related to the Surface Adhesion Protein CD36. Miguel A. Vega, Bartolome Segui-Real, Jose Alcalde Garcia, Carmela Cales, Fernando Rodriguez, Joel Vanderkerckhovev, and Ignacio V. Sandoval. THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 266(25): 16818-16824,1991), DEC-205 (see for example ~~SEQ ID NOS:14, 16, 34, 36~~ SEQ ID Nos. 13, 15, 33, and 35) (The Dendritic Cell Receptor for Endocytosis, DEC-205, Can Recycle and Enhance Antigen Presentation via Major Histocompatibility Complex Class II positive Lysosomal Compartments Karsten Mahnke, Ming Guo, Sena Lee, Homero Sepulveda, Suzy L. Swain, Michel Nussenzweig, and Ralph M. Steinman. The Journal of Cell Biology, Vol. 151(3): 673-683,2000; Efficient Targeting of Protein Antigen to the Dendritic Cell Receptor DEC-205.in the Steady State Leads to Antigen Presentation on Major Histocompatibility Complex Class I Products and Peripheral CD8+ T Cell Tolerance. Laura Bonifaz, David Bonnyay, Karsten Mahnke, Miguel Rivera, Michel C. Nussenzweig, and Ralph M. Steinman. J. Exp. Med. Vol. 196(12): 1627-1638, 2002; cDNA cloning of human DEC-205, a putative antigen-uptake receptor on dendritic cells. Masato Kato, Teresa K. Neil, Georgina J. Clark Christine M. Morris, Ru diger V. Sorg, Derek N.J. Hart. Immunogenetics, 47: 442-450, 1998), P-selectin (see for example ~~SEQ ID NOS:18, 38~~ SEQ ID Nos. 17 and 37) (Lysosomal Targeting of P-selectin Is Mediated by a Novel Sequence within Its Cytoplasmic Tail. Anastasia D. Blagoveshchenskaya, John P. Norcott, and Daniel F. Cutler. THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 273(5): 2729-2737, 1998; A Balance of

Opposing Signals within the Cytoplasmic Tail Controls the Lysosomal Targeting of P-selectin. Anastasia D. Blagoveshchenskaya, Eric W. Hewitt, and Daniel F. Cutler. THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 273(43): 27896-27903, 1998; Targeting of P-Selectin to Two Regulated Secretory Organelles in PC12 Cells. John P. Norcott, Roberto Solari, and Daniel F. Cutler. The Journal of Cell Biology, Vol. 134(5): 1229-1240, 1996; Structural and Functional Characterization of Monomeric Soluble P-selectin and Comparison with Membrane P-selectin. Shigeru I Ushiyama, Thomas M. Laue, Kevin L. Moore, Harold P. Erickson, and Rodger P. McEver. THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 268(20): 15229-15237, 1993; Structure of the Human Gene Encoding Granule Membrane Protein-140, a Member of the Selectin Family of Adhesion Receptors for Leukocytes. Geoffrey I. Johnston, Greg A. Bliss, Peter J. Newman and Rodger P. McEver. THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 265(34): 21381-21385, 1990), tyrosinase (see for example ~~SEQ ID NOS:20, 40~~ SEQ ID Nos. 19 and 39) (THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 274, No. 18, Issue of April 30, pp. 12780-12789, 1999. A Cytoplasmic Sequence in Human Tyrosinase Defines a Second Class of Di-leucine-based Sorting Signals for Late Endosomal and Lysosomal Delivery. Paul A. Calvo, David W. Frank, Bert M. Bieler, Joanne F. Berson, and Michael S. Marks), the glucose transporter GLUT4 (see for example ~~SEQ ID NOS:22, 42~~ SEQ ID Nos. 21 and 41) (The cytosolic C terminus of the glucose transporter GLUT4 contains an acidic cluster endosomal targeting motif distal to the dileucine signal. Annette M. Shewan, Brad J. Marsh, Derek R. Mmelvin, Sally Martin, Gwyn W. Ggoulda and David E. James. Biochem. J. 350: 99-107, 2000; Cloning and Characterization of the Major Insulin-responsive Glucose Transporter Expressed in Human Skeletal Muscle and Other Insulin responsive Tissues. Hirofumi Fukumoto, Toshiaki Kayanoh, John B. Busel, Yvonne Edwards, Paul F. Pilch, Graeme I. Bell, and Susumu Seino. THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 264(14): 7776-7779, 1989), endotubulin (see for example ~~SEQ ID NOS:24, 44~~ SEQ ID Nos. 23 and 43) (Cytoplasmic Signals Mediate Apical Early Endosomal Targeting of Endotubulin in MDCK Cells. K. E. Gokay, R. S. Young and J. M. Wilson. Traffic, 2: 487-500, 2001; Targeting of an Apical Endosomal Protein to Endosomes in Madin-Darby Canine Kidney Cells Requires Two Sorting Motifs. K.E. Gokay and J.M. Wilson. Traffic, 1: 354-365, 2000), or Nef protein or a functional equivalent meaning any variation in the sequence that does not affect its function of mediating translocation to

endoplasmic reticulum, for example allelic variants, conservative amino acid substitutions and substantially homologous sequences as described in more detail below. In one embodiment, the gene encodes an N-terminal signal sequence of LAMP-1 or a functional equivalent. In another embodiment, the gene encodes an N-terminal signal sequence of human tissue plasminogen activator or a functional equivalent.

Please replace paragraph no. 0036 with the following amended paragraph.

[0036] Preferably, the recombinant DNA further comprises an operably linked gene encoding a second signal peptide wherein the second signal peptide targets the allergen to an endosome or lysosome. This is believed to further enhance presentation of the allergen in the MHC class II pathway. The gene encoding the second signal peptide may be any sequence that encodes an amino acid sequence that interacts with the cell machinery to target the allergen to which it is attached to a lysosome or an endosome. For example and without limitation, the second signal peptide may be the C-terminal lysosomal/endosomal targeting sequence from the gene for LAMP-1, human tissue plasminogen activator, LIMP-II (see for example ~~SEQ ID NOS:9, 11, 13, 29, 31, 33~~ SEQ ID Nos. 8, 10, 12, 28, 30, and 32), DEC-205 (see for example ~~SEQ ID NOS:15, 17, 35, 37~~ SEQ ID Nos. 14, 16, 34, and 36), P-selectin (see for example ~~SEQ ID NOS:19, 39~~ SEQ ID NOS:18, 38), human tyrosinase (see for example ~~SEQ ID NOS:21, 41~~ SEQ ID Nos. 20 and 40), the glucose transporter GLUT4 (see for example ~~SEQ ID NOS:23, 43~~ SEQ ID Nos. 22 and 42), endotubin (see for example ~~SEQ ID NOS:25, 45~~ SEQ ID Nos. 24 and 44) or Nef protein, or a functional equivalent meaning any variation in the sequence that does not affect its function of targeting to an endosome or lysosome, for example allelic variants, conservative amino acid substitutions and substantially homologous sequences as described in more detail below. In one embodiment, the gene encodes the transmembrane and cytoplasmic domain of LAMP-1 or a functional equivalent.

Please replace paragraph no. 0044 with the following amended paragraph.

[0044] In one embodiment, the gene encoding the first signal peptide is operably linked upstream to the gene encoding the allergen and the gene encoding the second signal peptide is operably linked downstream from the gene encoding the allergen. In specific embodiments, the recombinant DNA comprises one or more sequences of ~~SEQ ID NOS. 3 to 7 and 28 to 48~~ SEQ ID Nos. 2 to 6 and 27 to 47.

Please replace paragraph no. 0096 with the following amended paragraph.

[0096] Plasmid pCI-LAMPss-Bt5₅₀₋₆₇-T/C was constructed using synthetic oligonucleotide composing the *Blo t 5* gene fragment that encodes for the H-2^d-restricted Th epitope. The oligonucleotide was inserted into the *Nhe I* site at the 3' end of the LAMP-1 leader sequence and the *Nde I* site at the 5' end of the LAMP-1 sequence encoding the transmembrane and cytoplasmic tail. The encoding sequence is ~~[SEQ ID NO:2]~~ SEQ ID No. 2:

Mouse LAMP-1 signal sequence (SEQ ID No. 2 (DNA); SEQ ID No. 25 (peptide))

M A A P G A R R P L L L L L A G L A H G A S

5' atggccgccccggcgcccgaggccctgctcctgctgctgctggcaggcctgcacatggcgctagc 3'

Blo t 5 H-2^d-restricted T cell epitope (SEQ ID No. 2 (DNA), continued; SEQ ID No. 49 (peptide))

A E L Q E K I I R E L D V V C A M N

5' gcagaattgcaagagaaaatcattcgagaacttgatgttgttgcgccatgaat 3'

Mouse LAMP-1 transmembrane & cytoplasmic domain (SEQ ID No. 2 (DNA), continued; SEQ ID No. 52 (peptide))

N M L I P I A V G G A L A G L V L I V L I A Y L

5' aatatgttgatccccattgctgtggcggtgccctggcagggtgtgtcctcatgctcctcattgcctacctc

~~Mouse LAMP-1 transmembrane & cytoplasmic domain~~

I G R K R S H A G Y E T I A M B
attggcaggaagaggagtcacgccggctatcagaccatctag 3 '

Please replace paragraph no. 0097 with the following amended paragraph.

[0097] Plasmid pCI-LAMPss- Bt5-T/C was generated by insertion of PCR amplified *Blo t 5* cDNA encoding the mature protein into the *Nhe I* site at the 3' end of the LAMP-1 leader sequence and the *Nde I* site at the 5' end of the LAMP-1 sequence encoding the transmembrane and cytoplasmic tail. The *Blo t 5*- LAMP I encoding sequence is ~~[SEQ ID NO:3];~~SEQ ID No. 3:

Mouse LAMP-1 signal sequence (SEQ ID No. 3 (DNA); SEQ ID No. 25 (peptide))
M A A P G A R R P L L L L L A G L A H G A S
5' Atggccgcccccggcgccggaggccctgctcctgctgctggcaggccttgacatggcgctagc 3'

Blo t 5 encoding sequence (SEQ ID No. 3 (DNA), continued; SEQ ID No. 50 (peptide))
Q E H K P K K D D F R N E F D H L L I E Q A N H
5' caagagcacaagccaaagaaggatgattccgaaacgaattcgatcacttggtgatcgaacaggcaaaccat
A I E K G E H Q L L Y L Q H Q L D E L N E N K S
gctatcgaaaagggagaacatcaattgctttacttgcaacaccaactcgacgaattgaatgaaaacaagagc
K E L Q E K I I R E L D V V C A M I E G A Q G A
aagggaattgcaagagaaaaatcattcgagaacttgatgttggtgcgccatgatcgaaggagcccaaggagct
L E R E L K R T D L N I L E R F N Y E E A Q T L
ttggaacgtgaattgaagcgaactgatcttaacatttgaacgattcaactacgaagaggctcaaactctc
S K I L L K D L K E T E Q K V K D I Q T Q ~~N~~
agcaagatcttgcttaaggatttgaaggaaaccgaacaaaaagtgaaggatattcaaaccctaaat 3'

Mouse LAMP-1 transmembrane & cytoplasmic domain (SEQ ID No. 3 (DNA),
continued; SEQ ID No. 52 (peptide))

N M L I P I A V G G A L A G L V L I V L I A Y L I
5' aatatgttgatccccattgctgtgggcgggtgccctggcagggtggtcctcatgctcctcatgcctacctcatt

G R K R S H A G Y E T I
ggcaggaagaggagtcacgccggctatcagaccatctag 3 '

Please replace paragraph no. 0098 with the following amended paragraph.

[0098] Plasmid pCI- LAMPss-Bt5 was derived from pCI-LAMPss-Bt5-T/C by replacement of the *Eco RI* /*Not I* fragment encoding for a portion of Blo t 5 and the LAMP-1 transmembrane and cytoplasmic domain with the *Eco RI*/*Not I* fragment from pCI-Bt5. The encoding sequence is ~~{SEQ ID NO: 4}~~ SEQ ID No. 4:

Mouse LAMP-1 signal sequence (SEQ ID No. 4 (DNA); SEQ ID No. 25 (peptide))
M A A P G A R R P L L L L L A G L A H G A S
5' Atggccgcccccggcgccggaggcccctgctcctgctgctgctggcaggccttgcatggcgctagc 3 '

Blo t 5 encoding sequence (SEQ ID No. 4 (DNA), continued; SEQ ID No. 50 (peptide))
Q E H K P K K D D F R N E F D H L L I E Q A N H
5' caagagcacaagccaaagaaggatgattccgaaacgaattcgatcacttggtgatcgaacaggcaaacat
A I E K G E H Q L L Y L Q H Q L D E L N E N K S
gctatcgaaaagggagaacatcaattgctttacttgcaacaccaactcgacgaattgaatgaaaacaagagc
K E L Q E K I I R E L D V V C A M I E G A Q G A
aaggaattgcaagagaaaatcattcgagaacttgatgtgttgcgccatgatcgaaggagcccaaggagct
L E R E L K R T D L N I L E R F N Y E E A Q T L
ttggaacgtgaattgaagcgaactgatcttaacattttggaacgattcaactacgaagggtcaaactctc

S K I L L K D L K E T E Q K V K D I Q T Q-N

agcaagatcttgcttaaggatttgaaggaaaccgaacaaaaagtgaaggatattcaaaccctaaattaa 3'

Please replace paragraph no. 0099 with the following amended paragraph.

[0099] Plasmid pCI-LAMPss-Derpl-T/C was generated by insertion of PCR amplified Der pl fragment encoding for the mature *Der p 1* protein (ref. 20. The gene bank access number is U11695) into the *Nhe I* site at the 3' end of the LAMP-1 leader sequence and the *Nde I* site at the 5' end of the LAMP-1 sequence encoding the transmembrane and cytoplasmic tail. The encoding sequence is {~~SEQ ID NO: 5~~} SEQ ID No. 5:

Mouse LAMP-1 signal sequence (SEQ ID No. 5 (DNA); SEQ ID No. 25 (peptide))

M A A P G A R R P L L L L L A G L A H G A S

5' atggccgccccggcgcccgaggccctgctcctgctgctgctggcaggccttgcatggcgctagc 3'

~~(+1)~~ mature *Der p 1* encoding sequence (SEQ ID No. 5 (DNA), continued; SEQ ID No. 51 (peptide))

(+1)

T N A C S I N G N A P A E A D L R Q M R T V T P I

5' actaacgcctgcagtatcaatggaaatgctccagctgaaatcgatttgcgacaaatcggaactgtcactccatt

R M Q G G C G S C W A F S G V A A T E S A Y L A Y

cgtatgcaaggaggctgtggtcatgttgggcttctctggtgtgccgcaactgaatcagcttattggcttac

R N Q S L D L A E Q E L V D C A S Q H G C H G D T

cgtaatcaatcattggatcttctgaacaagaattagtcgattgtgcttcccaacacggtgtcatggtgatacc

I P R G I E Y I Q H N G V V Q E S Y Y R Y V A R E

attccacgtggtattgaatacatccaataatggtgtcgtccaagaaagctactatcgatacgttgacgagaa

Q S C R R P N A Q R F G I S N Y C Q I Y P P N V N
caatcatgccgacgaccaaatgcacaacgtttcggtatctcaaactattgccaaatttaccaccaaagttaaac
K I R E A L A Q T H S A I A V I I G I K D L D A F
aaaattcgtgaagctttggctcaaaccacagcgctattgccgtcattattggcatcaaagatttagacgcattc
R H Y D G R T I I Q R D N G Y Q P N Y H A V N I V
cgtcattatgatggccgaacaatcattcaacgcgataatggttaccaccaaactatcacgctgtcaacattgtt
G Y S N A Q G V D Y W I V R N S W D T N W G D N G
ggttacagtaacgcacaaggtgtcgattattggatcgtagaaacagttgggataccaattgggggtgataatggt
Y G Y F A A N I D L M M I E E Y P Y V V I L ~~N~~ (+222)
tacggttattttgctgccaacatcgatttgatgatgattgaagaatatccatattgtgtcatttcaat3'

Mouse LAMP-1 transmembrane & cytoplasmic domain (SEQ ID No. 5 (DNA),
continued; SEQ ID No. 52 (peptide))

N M L I P I A V G G A L A G L V L I V L I A Y L I G
5' aatatgttgatccccattgctgtgggcgggtgccctggcagggtggtcctcatcgtcctcatcgcctacctcattggc
R K R S H A G Y E T I
aggaagaggagtcacgccggctatcagaccatctag 3 '